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Production and purification of high molecular weight oligomers of *Yersinia pestis* F1 capsular antigen released by high cell density culture of recombinant *Escherichia coli* cells carrying the *caf1* operon

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Background

Yersinia pestis fraction 1 antigen (F1) is the major component of the pathogen capsule. F1, a 15.5 kDa monomer that forms high molecular oligomers (>1000 kDa), is a highly protective antigen and is considered as a key constituent of a subunit anti-plague vaccine.

F1 antigen is encoded on the *caf1* operon together with a transcriptional regulator (*caf1R*), a chaperone (*caf1M*) and an usher protein (*caf1A*) and its expression is induced by a temperature shift to 37°C. Production and purification of F1 protein from *Y. pestis* cells is a tedious and time consuming procedure. Use of recombinant *Escherichia coli* for the production of F1 was reported [1,3].

Here, we describe an efficient procedure for production and purification of F1 released from the cell surface of *E. coli* cells, carrying the *caf1* operon, grown to a high cell density.

Results

The *caf1* operon, derived from *Y. pestis* Kimberley53 virulent strain [2], was cloned into the medium copy-number plasmid pBR322-Kanamycin (to yield pBRK-F1), and the expression plasmid was introduced into *Escherichia coli* MC1060. Under expression conditions the cells were found to produce high levels of F1 as a capsule (Y.L., E.M., S.C., Y.F., unpublished data).

Fermentation

Expression of F1 by *E. coli* MC1060 (pBRK-F1) was studied in a 4-liter computer-controlled fermentor using a rich medium devoid of animal products: soy protein extract, trace elements and glycerol as carbon source (SY). This medium enabled efficient bacterial growth to a high cell density (60 OD_{600}, Fig 1A). No loss of the expression plasmid was observed under these conditions. Unexpectedly, high and constant level of F1 expression was observed throughout the fermentation (up to 24 hours) even at 28°C (no temperature shift to 37°C was required, Fig. 1B). Moreover, at a high cell density (>10 OD_{600}), F1 was released from the encapsulated *E. coli* cells and accumulated up to 0.9 g/L in the culture medium (Fig. 1C).

Purification

Following centrifugation, the supernatant containing F1 was micro-filtered through 0.2 μm tangential flow cartridges with no apparent yield loss. Native PAGE and size exclusion chromatography analyses indicated a high molecular weight of F1 oligomers (>1000 kDa). Despite the oligomers size, F1 passed through ultra-filtration tangential flow cartridges having nominal pore size of 300

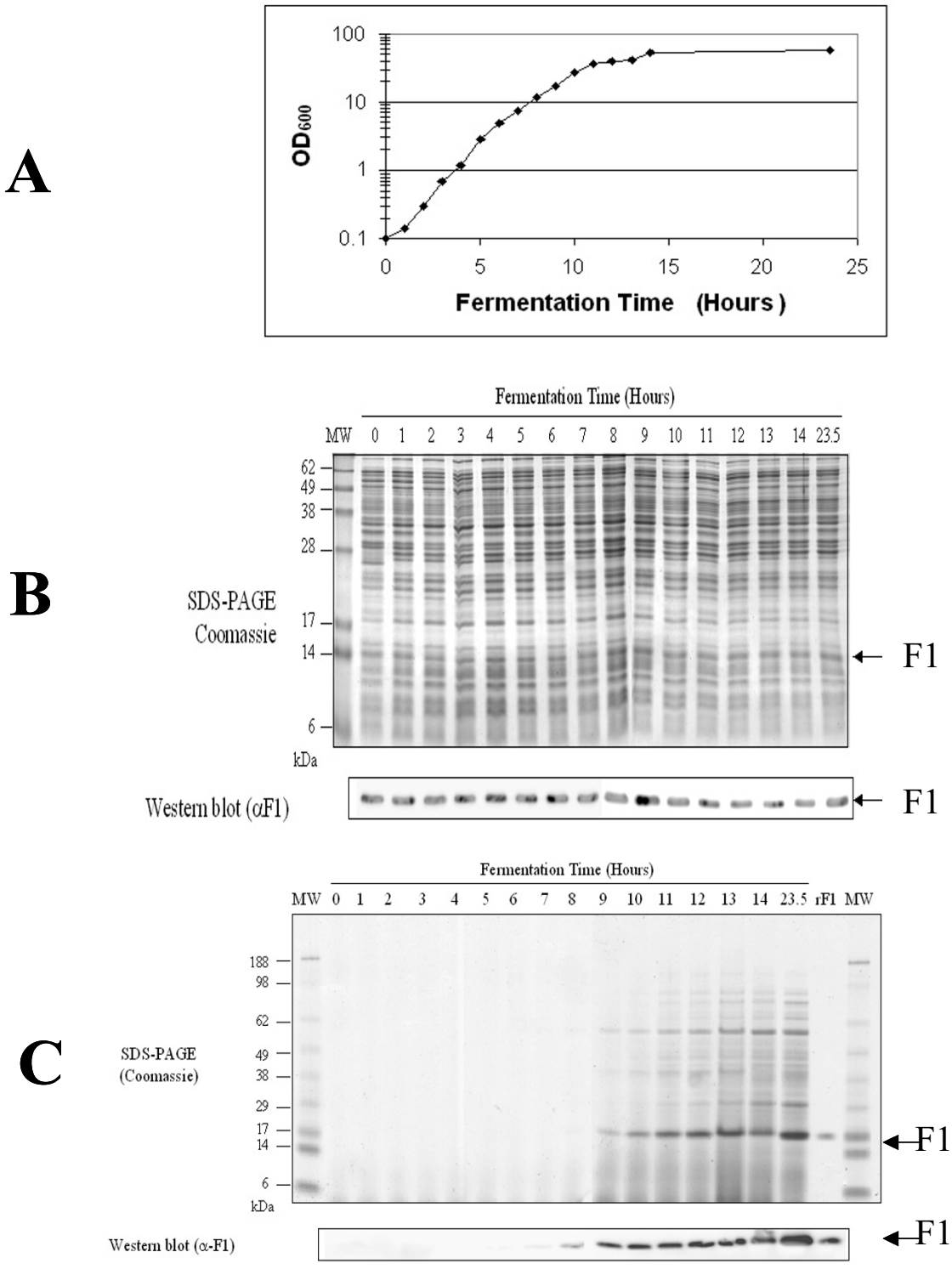

Figure 1
Expression and release of F1 protein by recombinant *E. coli* MC1060 (pBRK-F1) during fermentation. Culture growth was followed (A), and SDS-PAGE and Western blot analysis were performed on bacterial cell lysates (B, 0.05 OD_{600}/lane), and culture supernatant (C, 15 μl/lane).

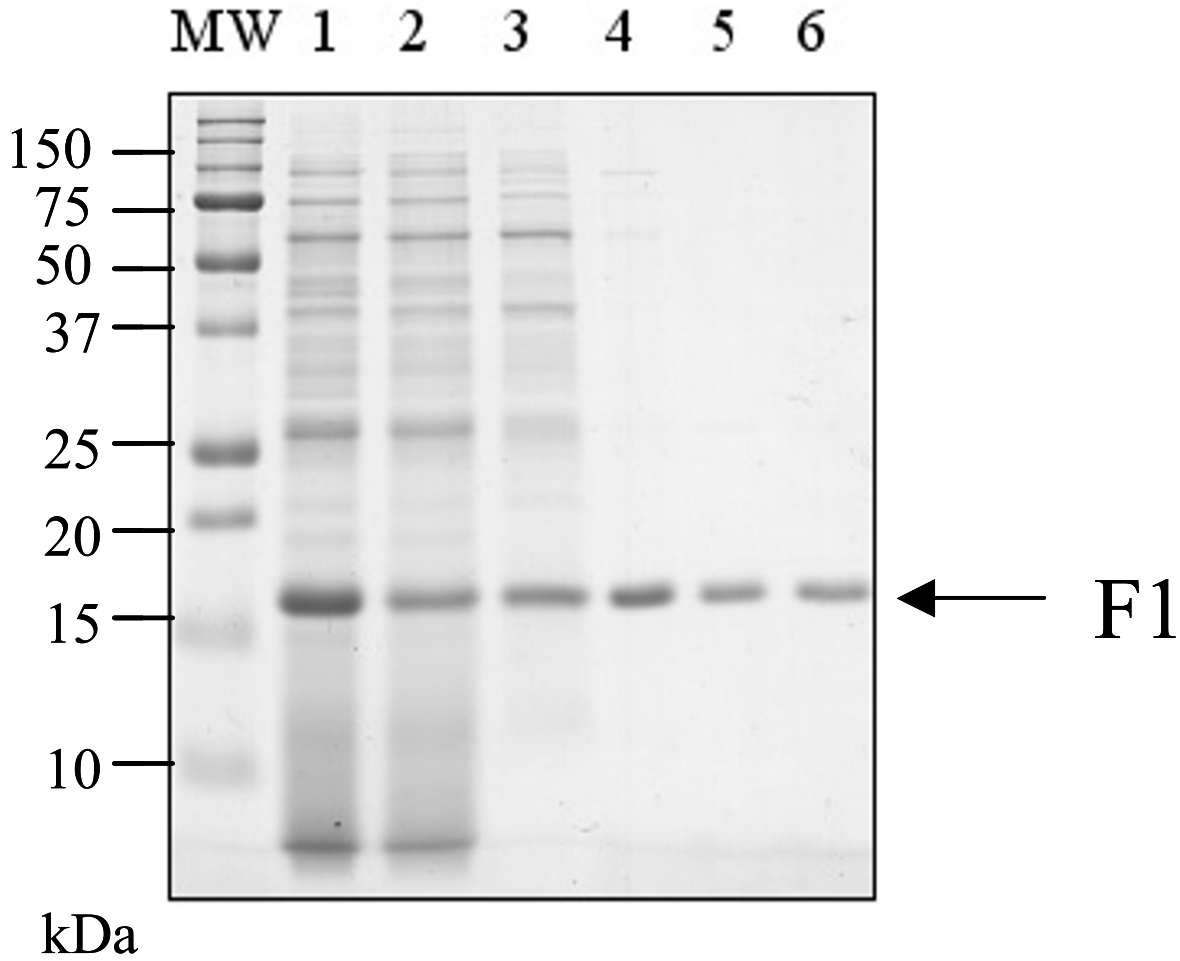

Figure 2
Purification process of rF1 antigen. (1) Fermentation culture supernatant. (2) 0.2 μm micro-filtration filtrate. (3) 50 kDa ultra-filtration retentate. (4) 33% ammonium sulfate precipitate. (5) Flow-through of Superdex-200 size-exclusion chromatography. (6) *Y. pestis* F1 standard.

and 100 kDa and could be finally concentrated using ultra-filtration cartridges of 50 kDa (Fig. 2).

Further purification steps were as follows: (a) 33% saturation ammonium sulfate precipitation, (b) size exclusion chromatography and (c) endotoxin removal resulting in at least 3-log reduction of endotoxin content, to ~200 EU/ml. The highly purified F1 antigen (>90%) consisted of high molecular oligomers with final recovery yield of 14% (125 mg F1/L culture).

Conclusion

1. E. coli cells expressing F1 could be grown to a high cell density in SY medium at 28°C, using a computer-controlled fermentor.

2. Continuous high level expression of F1 protein by recombinant *E. coli* was achieved during fermentation with no plasmid loss.

3. F1 antigen was massively released from the encapsulated cells at high cell density cultures.

4. High molecular weight oligomers of F1 were purified to >90% following few purification steps. This preparation was used for vaccination experiments in animal models.

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